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Research Article

# Essential oil profile and biological activity of the paleoendemic species *Salvia dorystaechas* B.T. Drew

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**Abstract:** *Salvia dorystaechas* B.T. Drew (syn. *Dorystaechas hastata* Boiss. & Heldr. ex Benth.) is an endemic plant native to Antalya and its surroundings, known as "Dağ çayı" and "Çalba Çayı". In the present study, *n*-hexane, ethyl acetate, ethanol 70% extracts and essential oil were obtained from the aerial parts of *S. dorystaechas.* The oil was obtained by the hydrodistillation method. The chemical composition of oil was determined by GC/FID and GC/MS analysis. Twenty-seven compounds were identified representing 99% of the oil. 1,8-cineole (26.4%), myrcene (19.2%) and α-pinene (10.1%) were determined as the main components. The extracts of the plant were screened for antioxidant activity by using a DPPH• free radical scavenging assay. The ordering of extracts in terms of antioxidant activity from highest to lowest was ethyl acetate, ethanol 70% and *n*-hexane. The extracts and hydrodistilled oil of *S. dorystaechas* were evaluated for their antimicrobial activity using standard broth microdilution protocols. The ethyl acetate extract exhibited the highest antibacterial and anticandidal activities against *S. aureus, S. typhimurium, C. utilis,* and *C. tropicalis*, with a minimum inhibitory concentration (MIC) of 62.5 µg/mL. The essential oil and the ethanolic extract demonstrated moderate to weak inhibitory effects (62,5 to >2000 µg/mL, MIC) against tested microorganisms. *S. dorystaechas* extracts demonstrate strong antimicrobial properties against various pathogens, suggesting potential use as a natural antibiotic, especially in light of increasing antibiotic resistance. Furthermore, the plant's aromatic components may be beneficial in aromatherapy. *S. dorystaechas* presents a promising candidate for natural therapeutic interventions, warranting further investigation into its pharmacological benefits.

## 1. INTRODUCTION

Türkiye is a country rich in plant diversity. The reason is that Türkiye has different climatic types, geological situations, geographical locations, topographic characteristics, and soil types and Anatolia has three different plant geographies region have (Davis, 1965). The family *Lamiaceae* is very rich in iridoids, alkaloids, and aromatic substances and includes about 232 genera and 8091 species in the world (Yıldız & Aktoklu, 2010; www.bizimbitkiler.org). The family *Lamiaceae*, which includes 46 genera in Türkiye, is the third largest family in terms of size with 592 species, 193 subspecies, and 42 varieties. The endemism rate is quite high, 326

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out of 844 taxa are endemic plants (www.wfoplantlist.org). Members of the family *Lamiaceae* are used both by the public and in medicine to treat many diseases. In addition to heart, vascular, gynecological diseases, and musculoskeletal disorders; It is also widely used in eye and mouth problems (Çatak & Atalay, 2022).

*Salvia* is the second largest genus in terms of the number of taxa (107 taxa) in the family *Lamiaceae* (Celep & Dirmenci, 2017). *Salvia* called "Sage", which has antioxidant, antibacterial, antidiabetic, and antitumor effects and more, has calming and anti-fatigue benefits and is used in the treatment of sore throat, colds, some cancers, and heart diseases (Karadal, 2022). The genus *Salvia* comprises approximately 1,050 species worldwide, 113 of which are found in Türkiye, with 58 being endemic (www.bizimbitkiler.org; www.wfoplantlist.org). *Salvia* has been claimed to be rich in essential oils and phenolic compounds, which is associated with its use in traditional medicine, pharmaceuticals, food, and cosmetics (Afonso *et al.*, 2019). *Salvia* (sage) species have been used in traditional folk medicine since ancient times as tea, ointment, tincture or extract, as an analgesic, expectorant, carminative, sedative, antiperspirant, externally as wound healer; and as medicinal plants used in the treatment of colds, bronchitis, tuberculosis, menstrual disorders, and stomach disorders. *Salvia* species primarily contain phenolic acids, flavonoids, terpenes, and terpenoids. These secondary metabolites have been reported to exhibit diverse biological effects such as antimicrobial, antifungal, antiseptic, analgesic, antioxidant, antispasmodic, antidepressant, antimutagenic, anticholinesterase, hallucinogenic, antidiabetic, anticancer, antihypertensive, anti-inflammatory, tuberculostatic, vasodilator, hypoglycemic and insecticidal activities (Elmas & Elmas, 2021).

*Dorystoechas hastata* Boiss. & Heldr. ex Benth. belongs to the family Lamiaceae and locally endemic, Eastern Mediterranean element plant that grows only in and around Antalya (Hedge, 1975; Güner *et al*., 2012). *D. hastata* is known as "Dağ çayı, Çalba çayı, Devren kekiği" among the inhabitant (Özcan *et al.*, 2016). It's an aromatic herb and its tea has a sharp taste, also fresh or dried leaves are consumed in the form of tea and used by locals as a medicinal drink against colds (Karagözler *et al*., 2008). *D. hastata* has a length of 40-100 cm, with pale roots measuring 18.9-29.5 cm. It features a deeply branched, woody, and globular stem, with lanceolate-hastate leaves measuring 2.2-3.5 x 5.1-8.7 cm, covered in dense hairs. The inflorescence is a spica that ranges from 6.8 to 13 cm in length. The calyx length increases as the flower (3.48-4.23 mm) develops into fruit (4.6-7.6 mm). The corolla is white and measures 4.3-6.9 mm in length. The pollen grains are isopolar, tricolporate, and measure 60 x 100 µm. The nutlets are light brown, bright, and measure 0.6-0.9 x 1.6-2.3 mm. This plant is classified as an aromatic shrub (Yılmaz, 2006). With the new classification made in 2017, *D. hastata* was transferred to the genus *Salvia* and its new name was *Salvia dorystaechas* B.T. Drew (Hedge, 1975; Drew *et al*., 2017).

This study aims to determine the chemical composition of essential oil and the antioxidant and antimicrobial activity of different three extracts of *S. dorystaechas*. While previous studies have explored the properties of *S. dorystaechas*, this research provides novel insights into its chemical composition and biological activities, highlighting its potential therapeutic applications and establishing a foundation for future investigations into its medicinal value.

## 2. MATERIAL and METHODS

### 2.1. The Plant Material

*S. dorystaechas* (Figure 1) was collected from Kemer/Antalya in Türkiye in June 2021. The plant material was identified by Yavuz Bülent KÖSE and the specimen is preserved in the Herbarium at Anadolu University, Eskişehir, Türkiye (Voucher specimen no: ESSE 15819).

**Figure 1.** *S. dorystaechas* (photo by Yavuz Bülent KÖSE).

### 2.2. Isolation of Essential Oil

The essential oil was obtained from the dried aerial parts of the plant. A total of 80 g of the appropriately sized material was weighed, and 10 times its weight in distilled water was added. The mixture was subjected to hydrodistillation for 3 hours using a Clevenger apparatus.

### 2.3. GC, GC/MS Analysis and Identification of Compounds

GC analyses were performed using an Agilent 6890N GC system. FID temperature was set to 300°C and the same operational conditions were applied to a triplicate of the same column used in GC/MS analyses. Simultaneous auto-injection was employed to obtain equivalent retention times. Relative percentages of the separated compounds were calculated from the integration of the peak areas in the GC-FID chromatograms. The GC/MS analysis was carried out with an Agilent 5975 GC-MSD system (Agilent, USA; SEM Ltd., Istanbul, Turkey). Innowax FSC column (60m x 0.25mm, 0.25µm film thickness) was used with helium as carrier gas (0.8 mL/min.). GC oven temperature was kept at 60°C for 10 min and programmed to 220°C at a rate of 4°C/min and kept constant at 220°C for 10 min and then programmed to 240°C at a rate of 1°C/min. The split ratio was adjusted to 40:1. The injector temperature was at 250°C. The interphase temperature was at 280°C. MS were taken at 70 eV. The mass range was from m/z 35 to 450.

The components of essential oils were identified by comparison of their mass spectra with those in the in-house Baser Library of Essential Oil Constituents, Adams Library (Adams, 2007) MassFinder Library (Hochmuth, 2008), Wiley GC/MS Library (McLafferty & Stauffer, 1989) and confirmed by comparison of their retention indices. These identifications were accomplished by comparison of retention times with authentic samples or by comparison of their relative retention index (RRI) to a series of n-alkanes. Alkanes were used as reference points in the calculation of relative retention indices (RRI) (Curvers *et al*., 1985). Relative percentage amounts of the separated compounds were calculated from FID chromatograms.

### 2.4. Extract Preparation

To prepare the extracts, 70% ethanol, *n*-hexane, and ethyl acetate were utilized on the species. The plant's aerial parts were chopped into small pieces. A 20-gram portion was weighed, and 250 mL of solvent was added. The samples underwent maceration in an orbital shaker at 150 rpm at room temperature for 48 hours. After filtration through filter paper, the solvents were evaporated using a rotavapor under reduced pressure. The 70% ethanolic extract was then placed in a lyophilizer and frozen at -20°C. The solvent-free extracts were stored at +4 ºC in a refrigerator until further use.

### 2.5. Antioxidant Activity

The antioxidant activities of extracts obtained from the aerial parts of *S. dorystaechas* with *n*-hexane, ethyl acetate, and 70% ethanol were evaluated by the DPPH$^{\bullet}$ free radical scavenging test procedure as described by Agiel *et al*. (2024).

### 2.6. Antimicrobial Activity

*S. dorystaechas* extracts were evaluated for their antibacterial activity against *Escherichia coli* NRRL B-3008, *Staphylococcus aureus* ATCC 6538, *Pseudomonas aeruginosa* ATCC 27853, *Salmonella typhimurium* ATCC 13311, *Serratia marcescens* NRRL B-2544, *Klebsiella pneumoniae* NCTC 9633. Anticandidal activity was also screened by using *Candida albicans* ATCC 10231 and ATCC 90028, *C. utilis* NRRL Y- 900, *C. tropicalis* ATCC 750, and *C. parapsilosis* ATCC 22019. Anticandidal and antibacterial tests were performed according to partly modified CLSI M27-A2 and M7-A7 reference protocols. Unlike the protocols, the initial solution of the essential oil and the extracts were prepared at the concentrations of 8 mg/mL in sterile DMSO (Dimethyl sulfoxide) where the standard agents were prepared following CLSI methods. Chloramphenicol (Sigma) was used as an antibacterial while Ketoconazole (Sigma-Aldrich) was used as a standard antifungal agent (Clinical and Laboratory Standards Institute, 2006; NCCLS, 2002).

## 3. RESULTS

### 3.1. Essential Oil Components

As a result of the hydrodistillation process, a sharp-scented, pale yellow essential oil was obtained. The yield of the essential oil was calculated to be 1.625%. Both GC and GC/MS were used to analyze the essential oil. Twenty-seven compounds representing 99.1% of the essential oil were characterized by 1,8-cineole (26.4%), myrcene (19.2%), and α-pinene (7.5%) as major constituents. Table 1 displays the chemical components of *S. dorystaechas* essential oil as determined by GC-GC/MS analysis.

**Table 1.** The chemical components of *S. dorystaechas* essential oil.

| RRI | Compounds | % | IM |
|---|---|---|---|
| 1032 | **α-Pinene** | **10.1** | $t_R$, MS |
| 1076 | Camphene | 3.8 | $t_R$, MS |
| 1118 | β-Pinene | 5.8 | $t_R$, MS |
| 1132 | Sabinene | tr | $t_R$, MS |
| 1159 | δ-3-Carene | 3.5 | $t_R$, MS |
| 1174 | **Myrcene** | **19.2** | $t_R$, MS |
| 1188 | α-Terpinene | 0.4 | $t_R$, MS |
| 1190 | Sylvestrene | 0.3 | MS |
| 1203 | Limonene | 4.0 | $t_R$, MS |
| 1213 | **1,8-Cineole** | **26.4** | $t_R$, MS |
| 1255 | γ-Terpinene | 0.7 | $t_R$, MS |
| 1280 | *p*-Cymene | 0.8 | $t_R$, MS |
| 1290 | Terpinolene | 1.4 | $t_R$, MS |
| 1452 | 1-Octen-3-ol | 0.2 | $t_R$, MS |
| 1532 | Camphor | 3.7 | $t_R$, MS |
| 1553 | Linalool | 0.7 | $t_R$, MS |
| 1591 | Bornyl acetate | 0.5 | $t_R$, MS |
| 1611 | Terpinene-4-ol | 1.1 | $t_R$, MS |
| 1612 | β-Caryophyllene | 3.7 | $t_R$, MS |
| 1684 | δ- Terpineol | 0.6 | $t_R$, MS |
| 1706 | α-Terpineol | 2.7 | $t_R$, MS |
| 1719 | Borneol | 3.4 | $t_R$, MS |
| 2008 | Caryophyllene oxide | 0.2 | $t_R$, MS |
| 2104 | Guaiol | 5.1 | MS |
| 2232 | Bulnesol | 0.5 | MS |
| 2250 | α-Eudesmol | 0.1 | MS |

| | | | |
|---|---|---|---|
| 2257 | β-Eudesmol | 0.2 | MS |
| | *Grouped compounds (%)* | | |
| | *Monoterpene hydrocarbons* | 50.0 | |
| | *Oxygenated monoterpenes* | 38.6 | |
| | *Sesquiterpene hydrocarbons* | 3.7 | |
| | *Oxygenated sesquiterpenes* | 6.1 | |
| | *Others* | 0.7 | |
| | **Total %** | **99.1** | |

RRI: Relative retention indices calculated against n-alkanes; %: calculated from the FID chromatograms; tr:Trace (<0.1 %). Identification method (IM): $t_R$, identification based on the retention times of genuine compounds on the HP Innowax column; MS, was identified based on computer matching of the mass spectra with those of the in-house Baser Library of Essential Oil Constituents, Adams, MassFinder and Wiley libraries and comparison with literature data.

### 3.2. Antioxidant Activity

As a result of the DPPH$^{\bullet}$ free radical scavenging activity assay performed on extracts, the highest antioxidant activity was observed in the ethyl acetate, ethanol %70 and *n*-hexane extract, respectively (Table 2).

**Table 2.** The ability of extract in scavenging DPPH$^{\bullet}$ radical.

| Extracts | DPPH$^{\bullet}$ $IC_{50}$ (µg/mL) |
|---|---|
| Ethanol 70% (SA) | 65.85±0.02 |
| *n*-hexane (SH) | 99.8 ±0.002 |
| Ethyl acetate (SEA) | 46.6 ±0.011 |
| Gallic acid (GA) | 4.8 |

### 3.3. Antimicrobial Activity

*S. dorystaechas* extracts were evaluated against six bacterial and five candidal reference strains. It was determined that the lowest MIC value (62,5 µg/mL) and the highest antibacterial effect against six bacterial strains were in ethyl acetate extract. *Staphylococcus aureus* ATCC 6538 and *Salmonella typhimurium* ATCC 13311 were determined as bacterial strains with the most susceptible to ethyl acetate extract. All extracts generally showed MIC in the range of 62,5-2000 µg/mL, against bacterial strains tested. MBC values range from 125 to 2000 µg/mL. The essential oil did not show any effect at the highest dose used. It only showed an inhibitory effect against *Salmonella typhimurium* at 1000 µg/mL. *Candida* species were inhibited at concentrations in the range of 62,5-2000 µg/mL. Most active extracts against *Candida* were found as *n*-hexane and ethyl acetate, in the range of 62,5-250 µg/mL. The most susceptible strains were *C. utilis* NRRL Y-900 and *C. tropicalis* ATCC 750 (Table 3).

**Table 3.** Antimicrobial screening of *S. dorystaechas* herbal extracts and essential oil (MIC, µg/mL).

| Microorganisms | H | EA | E | EO | S |
|---|---|---|---|---|---|
| *Escherichia coli* NRRL B-3008 | >2000 | >2000 | >2000 | >2000 | 1 |
| *Staphylococcus aureus* ATCC 6538 | 125[1] | 62.5[2] | 125[3] | >2000 | 4 |
| *Pseudomonas aeruginosa* ATCC 27853 | 500 | 500 | 500 | >2000 | 32 |
| *Salmonella typhimurium* ATCC 13311 | 125[4] | 62.5[5] | 125[6] | 1000 | 1 |
| *Serratia marcescens* NRRL B-2544 | 500 | 500 | >2000 | >2000 | 32 |
| *Klebsiella pneumoniae* NCTC 9633 | 500 | 500 | >2000 | >2000 | 8 |
| *Candida albicans* ATCC 10231 | 125 | 125 | 250 | >2000 | 0.125* |
| *C. utilis* NRRL Y-900 | 62.5 | 62.5 | 250 | 250 | 0.06* |
| *C. albicans* ATCC 90028 | 250 | 250 | 500 | >2000 | 0.06* |
| *C. tropicalis* ATCC 750 | 62.5 | 62.5 | 125 | 500 | 0.125* |
| *C. parapsilosis* ATCC 22019* | 125 | 125 | 250 | 1000 | 0.03* |

H: *n*-hexane extract, EA: Ethyl acetate extract, E: Ethanol 70% extract, EO: Essential oil, S: Chloramphenicol; *Ketoconazole; [1,2,3,4,5,6]: Minimal Bactericidal Concentrations (MBC [1]: 125 µg/mL; [2]: 500 µg/mL; [3]:2000 µg/mL; [4]: 2000µg/mL; [5]: 1000 µg/mL; [6]:2000 µg/mL)

## 4. DISCUSSION and CONCLUSION

### 4.1. Essential Oil Composition

The literature states that essential oils were extracted using hydrodistillation from the flower and leaf sections of *S. dorystaechas*. Essential oil components were analyzed by the GC and GC/MS method. The main components of floral essential oil are myrcene (19.37%), 1,8-cineol (14.30%), β-pinene (9.19%), α-pinene (8.49%) and β-caryophyllene (6.18%), while the main components of leaf essential oil myrcene (20.71%), 1,8-cineol (18.76%), β-pinene (12.51%), α-pinene (8.54%), bornyl acetate (7.28%) and terpinene-4-ol (6.19%) 11. In another study; Baser and Ozturk (1992) revealed the components of essential oils obtained from different parts (spikes, woody stems, leaves and flowering leafy) of *S. dorystaechas* by GC and GC-MS method. Essential oil compositions have been observed that vary according to the plant part obtained, the distillation method, and the place of collection. The main components were found to be 1,8-cineol, α-pinene, borneol, guaiol and camphor (Baser & Ozturk, 1992). In a study conducted in 2015, the components of essential oils obtained from the branches, leaves, and all aerial parts of *S. dorystaechas* were determined. The main components were determined as 1,8-cineol, α-pinene, borneol, guaiol and camphor. It was observed that the results of the analysis were compatible and consistent with the previous study by Baser and Ozturk (Kan *et al.*, 2015). In this study, twenty-seven compounds representing 99.1% of the essential oil were characterized by 1,8-cineole (26.4%), myrcene (19.2%) and α-pinene (7.5%) as major constituents. The analysis results were found to be compatible with previous studies. 1,8-cineole, myrcene, and α-pinene are prominent components of *S. dorystaechas* essential oil, recognized for their therapeutic potential in managing respiratory conditions, inflammatory diseases, pain relief, and anxiety reduction.

### 4.2. Antioxidant Activity

In this study, the antioxidant activity of *S. dorystaechas* extracts was tested using the DPPH free radical scavenging method. The highest antioxidant activity was observed in the ethyl acetate, ethanol 70%, and *n*-hexane extracts, with $IC_{50}$ values of 46.6 µg/mL, 65.85 µg/mL, and 99.8 µg/mL, respectively. These results indicate a strong antioxidant potential, especially in the ethyl acetate extract, aligning with the findings of Karagözler *et al*. (2008) on *S. dorystaechas*. In their study, the hot water extract exhibited the highest antioxidant activity with an $IC_{50}$ value of 6.17 ± 0.53 µg/mL, followed by diethyl ether, water, ethanol, and butylated hydroxytoluene extracts. Although the $IC_{50}$ values for our extracts were higher, the general trend supports the significant antioxidant activity within the genus.

Additionally, Erkan *et al*. (2011) also demonstrated that petroleum ether and methanol extracts of *S. dorystaechas* exhibited potent antioxidant effects, with the petroleum ether extract showing the highest activity due to its carnosic acid and carnosol content. While our study focuses on *S. dorystaechas*, the similar behavior of ethyl acetate in both studies suggests a common antioxidant potential within these taxa. Although not all previous studies used gallic acid as a standard, they consistently demonstrate that extracts from *Salvia* species possess substantial antioxidant activity. This corroborates our findings and further indicates that *S. dorystaechas* may serve as a valuable source of natural antioxidants.

### 4.3. Antimicrobial Activity

In a study conducted by Balkan Bozlak *et al*. in 2020, the antimicrobial effect of eighteen oils was tested against four bacteria and three fungi. Minimum inhibitory concentration and minimum bactericidal concentrations were found. Among the eighteen oils, no effective oil was found against *A.baumanii* and *S.aureus*. *C.glabrata* showed the highest resistance to all oils including *S. dorystaechas* (Balkan Bozlak *et al.*, 2021). While there was no effective oil against *A.baumanii* and *S.aureus* among the eighteen oils in this study; In this study, *n*-hexane (125 µg/mL), ethyl acetate (62.5 µg/mL), and ethanol (125 µg/mL) extracts of *S. dorystaechas* were

found to be active against *S. aureus* ATCC 6538. In essential oils, the antibacterial effect is low (MIC>2000 µg/mL).

The major compound identified in the plant, 1,8-cineole, is known for exhibiting antimicrobial activity against various Gram-positive and Gram-negative bacteria. It particularly exerts its antibacterial effect by promoting cell membrane permeabilization and inhibiting bacterial biofilm formation. Moreover, it has been reported that 1,8-cineole reduces virulence factors and biofilm formation in bacteria by suppressing the quorum sensing (QS) mechanism (Hoch *et al*., 2023). Since hexane is an appropriate solvent for dissolving non-polar compounds, the antimicrobial activity observed in the hexane extract can be explained by the presence of non-polar components of the essential oil, such as α-pinene and myrcene.

The essential oil and extracts of *S. dorystaechas* show promising potential for applications in medicine, cosmetics, the food industry, and agriculture. Their natural, effective, and safe profiles, along with their notable antimicrobial and antioxidant activities, align with the growing consumer demand for plant-based alternatives in these areas.

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## Declaration of Conflicting Interests and Ethics

The authors declare no conflict of interest. This research study complies with research and publishing ethics. The scientific and legal responsibility for manuscripts published in IJSM belongs to the authors.

## Authorship Contribution Statement

**Yavuz Bülent Köse** took part in the data analysis and proofreading. **Gökalp İşcan** and **Mine Kürkçüoğlu** participated in the experimental work and proofreading. **Zeynep Gülcan** participated in the experimental work, preparation of the manuscript, and data analysis.

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